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A NEW CRYPTIC SPECIES OF *TRICHOGRAMMA* (HYMENOPTERA: TRICHOGRAMMATIDAE) FROM THE MOJAVE DESERT OF CALIFORNIA AS DETERMINED BY MORPHOLOGICAL, REPRODUCTIVE AND MOLECULAR DATA

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Abstract.—A new species of *Trichogramma, T. kaykai,* is described from the deserts of southern California where it is a common egg parasitoid of the lycaenid butterfly *Apodemia mormo.* The new species is closely related to *T. deion,* the most common *Trichogramma* in western North America. It is distinguished from *T. deion* by morphological, allozymic and ITS2 sequence differences; the two also appear to be reproductively incompatible.

Key Words: *Trichogramma,* new species, allozymes, ITS2 DNA sequences

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Collections of parasitized eggs of the lycaenid butterfly *Apodemia mormo* (C. and R. Felder) on *Eriogonum inflatum* in the Mojave Desert in the spring of 1988 were commonly parasitized by a species of *Trichogramma.* This species was originally considered as a light color form of *T. deion* Pinto and Oatman, the most common *Trichogramma* in western North America (Pinto et al. 1986). This conclusion was questioned when typical *T. deion* was found at the same localities also parasitizing *A. mormo* eggs. Subsequent study showed that this form differed from *T. deion* by minor but consistent morphological traits, and by molecular differences (allozymes and ITS2 sequences). In addition, strains of the two proved to be reproductively incompatible in the laboratory. Because this new species continues to be the subject of various ecological and cytological studies (e.g. Stouthamer and Kazmer 1994) it is important that it receive a formal name. The species description below is followed by a brief summary of crossing results, and allozymic and DNA sequence comparisons. The molecular data are compared among the new species, *T. deion, T. pretiosum* Riley and the Interior form of *T. platneri* Nagarkatti. All taxa are similar morphologically and occur in sympatry in the Mojave Desert.

Species Description

The description is based on an examination of material from all localities comprising the range of the new species. The majority of this material consists of wasps individually mounted on glass slides in Canada balsam. For body length and color several specimens of both sexes [reared at ca. 24° C. and on eggs of *Trichoplusia ni* (Hübner)], and originating from two localities, were critically point dried after being killed in ethanol and mounted on cards. The material of *T. deion* compared in the diagnosis was reared under similar conditions.

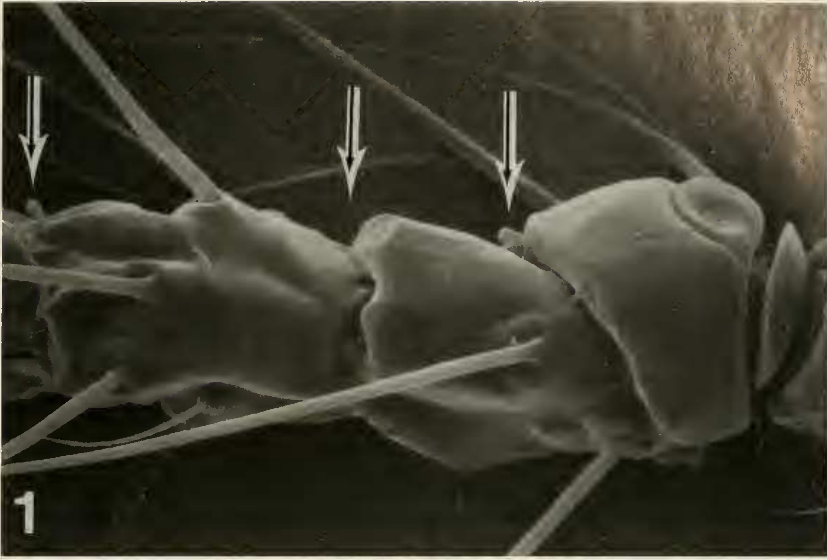


Fig. 1. Antenna of *Trichogramma kaykai* male (ventral of left antenna) showing base of flagellum (1500×). Arrows point to positions 1-3 (from right to left) showing presence of a single basiconic peg sensilla at positions 1 and 3 only. In *T. deion*, all three positions have a single sensilla.

Unless indicated, quantitative data (means ± SD and ranges) are taken from five randomly selected males, all from different localities. The hind tibial length of these specimens ranged from 0.16–0.19 mm. The terminology used in the description follows Pinto (1992).

Trichogramma kaykai Pinto and Stouthamer, new species

Trichogramma sp. near *deion*: Stouthamer and Kazmer, 1994:317.

Trichogramma sp.: Pinto and Stouthamer, 1994:23 (Table 1.1).

Color sexually dimorphic. Male darker with mesosoma including coxae orange brown to yellow brown; pronotum and mesoscutum typically darkest; metasoma primarily light brown; propodeum and posterior part of scutellum typically lightest, yellow orange in color. Female considerably lighter than male. Most of meso- and metasoma uniformly yellow orange; only pronotum, pro- and mesocoxa and ovipositor suffused with brown; anterior 2–3 metasomal terga lightly suffused with brown in some specimens.

Body length 0.4–0.5 mm in males, 0.5–0.6 mm in females.

Forewing 0.28 ± 0.02 mm wide, 0.54 ± 0.01 as wide as long, setation moderately dense with 20–29 setae between 4th and 5th setal tracks, longest fringe setae 0.14-0.20 maximum forewing width. Hind wing with 3–4 and 6–8 setae in anterior and posterior tracks, respectively, the latter attaining 0.5–0.6 the distance from hamuli to apex of wing. Scutellum with anterior pair of setae short, fine, ca. 0.2 the length of posterior pair.

Male.—Antenna with flagellum 0.17 ± 0.01 mm in length, slightly curved, 6.03 ± 0.24 as long as wide, 1.00 ± 0.04 as long as hind tibia, 2.1 ± 0.05 as long as scape; flagellar setae elongate, gradually tapering to apex, the longest of these setae 3.18 ± 0.21 (3.0–3.5) the basal width of flagellum; basiconic peg sensilla (BPS) relatively small, only slightly expanded apically, formula 1-0(1)-1-0-1-1 (i.e. position 2 usually lacking a sensilla as in Fig. 1, also see below for explanation); flagellum lacking unsocketed setae.

Genital capsule (GC) (Figs. 2, 3) mod-

erately broad, 0.34 ± 0.02 as wide as long; sides broadly constricted at level of intervolsellar process (IVP); parameres relatively straight, moderately and evenly convergent to apex; apical distance (between apex of parameres and base of IVP) 0.29 ± 0.01 total GC length; apical width (at base of IVP) 0.63 ± 0.2 greatest width of GC; dorsal aperature (DA) relatively elongate, narrowing considerably posteriorly, its length 0.63 ± 0.03 that of GC; dorsal lamina (DLA) arising slightly anterior to middle of GC, moderately notched at base and narrowing directly posterior to notch forming shoulders which usually do not approach sides of GC; posterior extension of DLA relatively elongate, linguiform, its width at level of IVP subequal to aedeagus width; DLA length from apex of DA = 0.79 ± 0.10 the apical distance and occupying 0.5–0.6 of this distance, usually extending slightly beyond apex of volsellae (VS); VS slightly bowed, occupying 0.4-0.5 the apical distance; IVP narrowly triangular, moderately elongate, occupying 0.3-0.4 the apical distance, its apex usually slightly anterior to that of VS; ventral processes positioned at base of IVP, slightly protuberant; ventral ridge relatively short and indistinct, occupying ca. 0.3 the distance from the base of the IVP to the base of GC. Aedeagus length subequal to that of GC and 0.70 ± 0.03 that of the hind tibia; apodemes consisting of ca. 0.5 entire aedeagus length.

Female.—Antenna with funicle segments subquadrate; usually with 1 BPS on the first funicular segment; second funicular segment usually lacking BPS. Ovipositor subequal in length to hind tibia (see below).

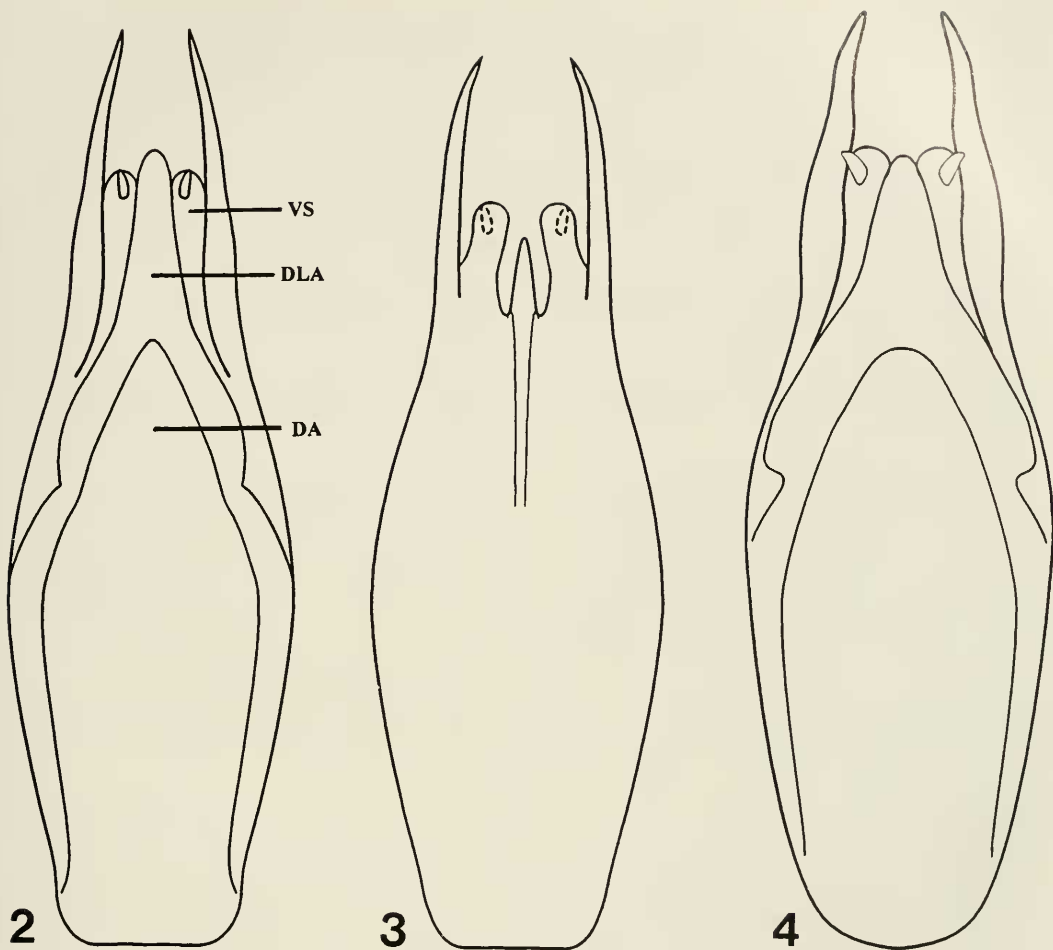
Types.—Holotype ♂ and allotype ♀ from CALIFORNIA, San Bernardino Co., Sheephole Pass, 3 mi. N. on Amboy Rd.; ex. *Apodemia mormo* on *Eriogonum inflatum* Torr. & Frém.; iv-30-93; K. Cooper, J. Pinto & G. Platner, collrs.; in the National Museum of Natural History, Smithsonian Institution, Washington, D. C. Eight ♂ and 3 ♀ paratypes with same data as primary type deposited in collections of the Canadian National Collection, Ottawa; University of California, Riverside; University of California, Berkeley; and The Natural History Museum, London. All type specimens are F1-F7 generation individuals from a culture started with a single female.

Etymology.—The specific name is an arbitrary combination of letters, treated as a noun in the nominative singular and faithful to "KK," the informal epithet applied to this species in our laboratory for several years.

Diagnosis.—*Trichogramma kaykai* is morphologically most similar to *T. deion* and *T. pretiosum.* All three have similar genitalic structure and relatively elongate setae on the male antenna. Males of *T. kaykai* and *T. deion* are separated from *T. pretiosum* by the more distinctly sclerotized posterior extension of the dorsal lamina and the BPS formula on the antenna. Unlike *T. kaykai* and *T. deion, T. pretiosum* has a pair of BPS at the second and third positions. Differences in this trait and male genitalia in *T. deion* and *T. pretiosum* are summarized in Pinto et al. (1986).

Trichogramma kaykai is separated from *T. deion* by color, minor but consistent differences in the male genitalia, BPS formula, and ovipositor length. Color in *T. deion* is not obviously sexually dimorphic as it is in *T. kaykai.* In *T. deion* the mesoscutum and metasoma of both sexes are typically brownish. Males of *T. kaykai* are similar but usually a lighter brown; females, however, are almost uniformly yellow or yellow orange and are easily separated from *T. deion* on this basis.

In most *Trichogramma* there are one or two basiconic peg sensilla (BPS) at each of the first three positions of the male flagellum. The presence of these sensilla are relatively stable within species, although they may be reduced or completely absent at these positions in abnormally small individuals. *T. deion* almost always has a single BPS at the first three positions. In *T. kaykai* a single sensilla is present at positions 1 and 3 but usually not at position 2 (Fig. 1). This



Figs. 2–4. Genital capsule of male (posterior end above). 2, *Trichogramma kaykai* (dorsal view), DLA = dorsal lamina, DA = dorsal aperature, VS = volsella. 3, *T. kaykai* (ventral view). 4, *T. deion* (dorsal view). Note in *T. kaykai* the DA is narrower apically, and the DLA is narrower at its base (immediately posterior to notch) and extends beyond the apex of the VS.

feature is variable, however. Whereas the formula in *T. deion* is relatively stable, about 35% of 135 non-sib males of *T. kaykai* examined had a sensilla at this position. Females of *T. kaykai* can also usually be separated from *T. deion* by the absence of a BPS at the equivalent antennal position, i.e., the apex of the second funicular segment.

Most males of *T. kaykai* can be separated from *T. deion* by genitalia structure (*cf.* Figs. 2, 4). In *T. kaykai* the genital capsule is narrower apically, the dorsal lamina is narrower immediately posterior to its basal notch, and, most importantly, the dorsal aperature is somewhat longer and narrows considerably at its posterior end. Correlated with this difference, the length of the dorsal lamina, measured from the posterior border of the dorsal aperature to its apex, is less in *T. kaykai* and averages 0.79 the apical distance; in *T. deion* this ratio averages 0.93 the apical distance. Also, in *T. kaykai* the lamina typically extends to a level slightly posterior to the apex of the volsellae; it usually does not extend that far in *T. deion.*

Females of *T. kaykai* are most easily distinguished by color. However, the longer

ovipositor also helps distinguish it from *T. deion.* Samples of 10 females of each species from localities where the two were sympatric were compared. All originated from different host eggs and had a hind tibial length ranging from 0.19–0.21 mm. In *T. kaykai,* the ratio of ovipositor to hind tibial length was 1.00 ± 0.03; in *T. deion* this ratio was 0.93 ± 0.04. Although the range of variation does overlap, this feature used with the significantly lighter coloration and absence of a BCP on the second funicular segment should provide straightforward separation of females.

Geographic distribution.—*Trichogramma kaykai* has only been collected in southern California -in the Mojave Desert and at the northern limits of the Sonoran Desert.

Hosts.—The primary host of this species is the egg of *Apodemia mormo* (Lycaenidae) laid on *Eriogonum inflatum.* This lycaenid has been divided into several subspecies (Miller and Brown 1981). All records of *T. kaykai* are from *A. m. deserti* Barnes and McDunnough. *Trichogramma kaykai* also has been taken a single time (Pinyon Mt., Kern Co.) on the egg of another lycaenid, *Icaricia lupini* (Boisduval), on *Eriogonum fasciculatum* Bentham.

Material examined.—401 specimens of both sexes. The material examined includes specimens emerging from field collected host eggs and from cultures initiated with these parentals.

Records.—Except for the single record from Pinyon Mt. (see Hosts), all following collections originated from the eggs of *Apodemia mormo* on *Eriogonum inflatum.* UNITED STATES. **California**. *Kern Co.* El Paso Mts. (Bickel Camp); vii-29-96; R. Stouthamer. Pinyon Mt.; vi-4-87; G. Pratt. Walker Pass, 2 mi. E.; iv-19-89; G. Pratt. Last Chance Cyn.; v-14-88; D. Kazmer, R. Stouthamer; also numerous collections by several collectors from iv-vi-95/96. Randsburg; v-14-88; D. Kazmer, R. Stouthamer. *Riverside Co.* Dillon Rd., several collections between immediately east of Indio and Fun Valley (all thelytokous); iv-26-95; J. Pinto, R. Stouthamer. *San Bernardino Co.* Barstow, ca. 10 mi. NE., and ca. 8 mi. E.; v-29-96, v-15-96; L. Bolijn, B. Deijkers. Beacon Station; vi-6-96; L. Bolijn, B. Deijkers. Danby; iv-11-88, iv-30-88, iv-27-95; G. Pratt/D. Kazmer/R. Stouthamer. Joshua Tree; vi-15-96; L. Bolijn, B. Deijkers. Hwy. 66 between Bagdad and Siberia; iv-27-95; R. Stouthamer. Interstate 15, between Victorville and Barstow; v-29-96; L. Bolijn, B. Deijkers. Kramer Hills; v-14-88, iv-24-95; D. Kazmer, R. Stouthamer/J. Bennett, R. Stouthamer. Lucerne Valley, ca. 12 mi. N.; v-29-96; L. Bolijn, B. Deijkers. Sheephole Pass area; v-14-88, iv-27-95; D. Kazmer, R. Stouthamer. Sheephole Pass, 3 mi. N. (on Amboy Rd.) (type locality); iv-30-93; K. Cooper, J. Pinto, G. Platner. Yucca Valley; vi-1-96; L. Bolijn, B. Deijkers.

Notes.—Both thelytokous and arrhenotokous populations of *T. kaykai* have been collected. As in many species of *Trichogramma,* thelytoky in this species is caused by *Wolbachia* infection and can be cured with antibiotic treatment (Stouthamer et al. 1990). Populations with both modes of reproduction occur together in the Mojave Desert. The few collections from the Sonoran Desert are all thelytokous.

Unlike *T. deion* which has very broad host and geographic ranges in western North America (Pinto et al. 1986), *T. kaykai* has been retrieved almost totally from eggs of *Apodemia mormo* in the deserts of southern California. Both species are known to occur together on *Apodemia* at several localities, although *T. kaykai* is more common and appears to be the dominant egg parasite of this butterfly in the Mojave Desert. Of a sample of 256 parasitized *A. mormo* eggs collected at several sites of sympatry during the spring of 1995, 212 or 82.9% were attacked by *T. kaykai*; only 38 or 14.8% were parasitized by *T. deion.* The remainder (2.3%) were attacked by a third and undescribed species. In a few cases, *T. deion* and *T. kaykai* emerged from the same egg.

The eggs of *A. mormo* are relatively large and several *Trichogramma* typically emerge from a single egg. In a sample of 33 eggs collected in 1988 from several localities the average number of *T. kaykai* emerging from each was 4.6 ± 1.2 (range = 1–7). Most of the progeny were female; most parasitized eggs result in a single male and 3–4 females. The average number of males emerging from this sample (excluding eggs resulting in thelytokous wasps) was 1.19 ± 0.75 (range = 0–3, n = 31).

Collections of other species of host at sites where *T. kaykai* occurs are minimal. The only such collection was made on 14 May 1988 at Last Chance Canyon in Kern County. The eight parasitized eggs of *A. mormo* collected on *Eriogonum inflatum* were attacked by both *T. deion* (5) and *T. kaykai* (3). However, only *T. deion* emerged from 23 parasitized eggs of an undetermined Pieridae collected from a species of *Stanleya* (Brassicaceae).

MOLECULAR DATA

Allozymes

An earlier paper compared allozymes in *T. pretiosum* and *T. deion* (Pinto et al. 1993) and reported consistent allelic differences between these close relatives. For this study we examined ten loci in four cultures of *T. kaykai,* and compared them with one culture of the interior form of *T. platneri* Nagarkatti, two cultures of *T. pretiosum* and seven of *T. deion.* All exemplars were run concurrently. The cultures chosen of the latter three species represented most of the known allelic diversity in *Trichogramma* at the loci examined. The enzyme systems used are as follows: Aconitase (4.2.1.3), *Acon*; acid phosphatase (3.1.3.2), *Acp-II*; fumarase (4.2.1.2), *Fum*; *a*-glycerolphosphate dehydrogenase (1.1.1.8), *aGpd-II*; glucose-phosphate isomerase (5.3.1.9), *Gpi*; glucose-6-phosphate dehydrogenase (1.1.1.49), *G6pd*; isocitrate dehydrogenase (1.1.1.42), *Idh*; malate dehydrogenase (1.1.1.37), *Mdh-II*; malic enzyme (1.1.1.40), *Me*; and phosphoglucomutase (2.7.5.1), *Pgm.* These loci, among others, were also compared between *T. pretiosum* and *T. deion* in our earlier study.

The four *T. kaykai* cultures examined were from Walker Pass (KWPA), Last Chance Cyn. (KLC187), and between Bagdad and Siberia (KRB85), three Mojave Desert localities, and Dillon Rd. (N. of Indio) (KAW73), a Sonoran Desert site. The cultures of *T. deion* examined represent much of the range of the species in western North America. The origin of the comparison cultures were as follows: *T. pretiosum* — *R*iverside, CA (PRV4) and Wyndham, Australia (PAWD). Interior form of *T. platneri*—Mesquite, NV (IMSQ). *T. deion* — Riverside, CA (DRV4); Seven Pines, CA (DSVP); Covelo, CA (DCLO); Portal, AZ (DPTL); Granite Gap (Hidalgo Co.), NM (DGGP); Miles City, MT (DMCT); Paul's Place (Kern Co.), CA (DPPL); and Last Chance Cyn (DLC1). All but two of these comparison cultures (DPPL and DLC1) also were examined in our previous paper (Pinto et al. 1993). The culture IMSQ was originally assigned to the interior form of *T. platneri* (Pinto et al. 1992). This form represents a new species and will be described in the near future. Until then we continue to refer to it as before.

All cultures compared represented isofemale lines. One culture of *T. kaykai* (KAW73) was originally thelytokous; all others were arrhenotokous. In the latter case, the female used to initiate a culture had mated with a brother.

Electrophoretic analysis followed methods in our earlier studies (Pinto et al. 1992, 1993) and were originally detailed by Kazmer (1991). Briefly, two females per culture were individually analyzed at each locus by isoelectric focusing in one to two layers of cellulose acetate membranes using a single blend of carrier ampholytes (8% pH 4–6.5 and 2% pH 3–10 pharmalytes) (Sigma Chemical, St. Louis, MO) and an effective gel length of 4.5 cm. Each culture represented an isofemale line initiated from a

Table 1. Allelic comparison of *Trichogramma kaykai* with closely related species at eight loci.[a,b]

		Loci and Alleles							
Taxon	Culture[c]	*AcpII*	*Fum*	*Gpi*	*aGpdII*	*G6pd*	*Idh*	*MdhII*	*Pgm*
Interior form	IMSQ	D	C	B	A	A	A	C	E
pretiosum	PRV4	D	B	B	A	B	B	A	D
	PAWD	D	B	B	A	B	B	E	E
deion	DRV4	C	B	B	B	A	A	C	B
	DSVP	D	B	A	A	A	B	C	C
	DCLO	C	B	B	A	A	B	C	B
	DPTL	D	B	B	A	A	A	B	C
	DGGP	D	B	B	A	A	B	C	B/C
	DMCT	D	B	B	B	A	A	C	A
	DPPL	C	B	B	A	A	B	C	B
	DLC1[d]	—	—	B	—	A	—	C	—
kaykai	KWPA	E	B	C	A	C/F	B	A	B
	KRB85	D	B	C	A	D/E	B	A	B
	KLC187	D	C	D	A	D/E	B	A	B
	KAW73	D	B	C	A	C/E	B	A	B

[a] Relative distances among electromorphs for the three loci distinguishing *T. kaykai* [distances for others given in Pinto et al. 1993)], based on ratio of distance between cathode and homomeric band to entire gel length, as follows (alleles in alphabetical order): *Gpi* (0.18, 0.46, 0.62, 0.67), *G6pd* (0.03, 0.11, 0.19, 0.23, 0.27, 0.32), *MdhII* (0.31, 0.38, 0.47, 0.56).

[b] All females examined were homozygous at all loci. Those entries showing two alleles indicate the two females examined were homozygous for different alleles.

[c] See text for geographic origin of cultures.

[d] DLC1, a *T. deion* collection sympatric with the *T. kaykai* KLC187, was only examined at the three loci distinguishing the two species.

single parasitized host egg collected in the field and maintained in the laboratory for several generations on eggs of *Trichoplusia ni*. BIOSYS-1 (Swofford and Selander 1989, release 1.7) was used to analyze data using individual genotypes as input.

Results.—Two loci (*Acon* and *Me*) were fixed in all samples examined. The remaining eight were polymorphic and are compared for all cultures in Table 1. The four *T. kaykai* cultures differed from all heterospecifics at two loci, *Gpi* and *G6pd*. One of these loci, *G6pd*, is the only one providing complete separation of *T. deion* and *T. pretiosum* (Pinto et al. 1993). The three allozymic differences between *T. kaykai* and *T. deion* also were found in one pair of sympatric collections (from Last Chance Cyn., Kern Co., CA). Cluster analysis based on the data in Table 1, using UPGMA clustering of Nei genetic distances, recognized three groups, all consistent with species identity. IMSQ joined closest to the *pretiosum* cultures. Mean Nei genetic distances between *T. kaykai* and *T. deion*, and between *T. kaykai* and *T. pretiosum* were 0.697 ± 0.24 (0.379–1.178, n = 27) and 0.490 ± 0.13 (0.311–0.668, n = 8), respectively. The mean intraspecific distance for *T. kaykai* was 0.218 ± 0.14 (0.027–0.460, n = 6). The interspecific distances are considerably greater than those reported in our earlier studies of closely related *Trichogramma* and are certainly exaggerated. This is because cultures of both *T. deion* and *T. pretiosum* were chosen for allelic diversity to insure detection of any differences that occurred in the new species.

The presence of at least two alleles of *G6pd* in the cultures of *T. kaykai* (Table 1) requires further investigation. Although this could be explained if the female initiating each culture was heterozygous, it is also possible, and perhaps more likely, that two loci for this enzyme are involved.

Table 2. The size of the ITS2 gene and size of the restriction fragments generated by restriction enzymes MSE1 and ECOR1.

Taxon	Culture[a]	Size ITS2 (bp)	MSE1 restr. fragments (bp)	ECOR1 restr. fragment (bp)
T. deion	DSVP	398	294, 61, 43	398
	DCLO	402	296, 63, 43	402
	DLC1	406	300, 65, 41	406
T. kaykai	KLC187	470	271, 199	470
	KSH1	463	263, 200	463
T. pretiosum	PRV4	409	409	409
	PIRV	413	413	413
Interior form	IMSQ	515	409, 81, 25	325, 190

[a] See text for geographic origin of cultures.

ITS2 DNA Sequences

The ribosomal nuclear genes (rRNA) are among the several genetic sequences proposed for distinguishing closely related species in insects (Hoy 1994). The ITS2 sequence, or Internal Transcribed Spacer region, is positioned between the 5.8S and 28S coding region of the rRNA gene. This sequence shows considerable promise in separating closely related species of *Trichogramma* (van Kan, et al. 1996). In this study we compared the complete ITS2 sequences of two *T. kaykai* cultures, three *T. deion* and two *T. pretiosum* cultures, and one culture of the interior race of *T. platneri* (see Table 2). Each culture represented an isofemale line initiated with a single parasitized host egg collected in the field and maintained for a variable number of generations on eggs of either *Trichoplusia ni*, *Mamestra brassicae* L. or *Ephestia kuehniella* (Zeller). Differences in sequence and size of the ITS2 gene were determined. Restriction enzymes were used to find characteristic differences among species. Cultures studied are indicated in Table 2. Several of these were also used for allozymes (Table 1). Those examined for ITS2 only include the following: *T. kaykai*—Last Chance Cyn., CA (KLC187); Sheephole Pass area (San Bernardino Co.) (KSH1). *T. pretiosum*—Irvine, CA (PIRV). Cultures included both allopatric and sympatric (DLC1 & KLC187) representatives of *T. deion* and *T. kaykai.*

The method for determining the ITS2 sequence, briefly, is as follows: One to three wasps, preserved in absolute ethanol, were ground in 50–150 μl 5% Chelex and 3 ul proteinase K (20 mg/ml) and incubated for at least 2 hrs. at 56°C followed by 10 min at 95°C. The wasps were first shaken in 1 ml TAE for 1 hr prior to grinding in Chelex. PCR was performed in 50 μl reaction volumes using a Hybaid thermocycler, 5 μl DNA template, 5 μl PCR-buffer, 1 μl d-NTP's (each in a 10mM concentration), 0.6 μl forward and reverse primer (10ng), 0.1 μl SuperTth polymerase enzyme (5 units/ul) from Spaero-Q; and 38 μl sterile distilled water. The ITS-2 region was amplified using the following primers; forward: 5'-TGTGAACTGCAGGACACATG-3'; reverse: 5'-AATGCTTAAATTTAGGGGGTA-3'. The PCR cycling program was 3 min. 95°C, 45 sec. at 53°C and 45 sec. at 72°C with 3 min. at 72°C after the last cycle. The machine was set to tube control. PCR products of about 550 bp were electrophoresed and excised from the agarose gel. They were then frozen and freeze-squeezed. The liquid phase was alcohol precipitated, washed and ligated into a T-tailed vector (Amersham Life Science) and amplified in *E. coli* cells. *Escherichia coli* colonies containing an insert of the correct size were checked by PCR using the primers mentioned above and were subsequently sequenced using an automatic sequencer (373 DNA Sequencer Stretch, Applied Biosys-

tems using a Prism Ready Reaction DyeDeoxyô Terminator Cycle sequence kit). The size of digestion products of the ITS2 gene using different restriction enzymes was determined. Characteristic differences among the species were found using ECOR1 and MSE1.

Results.—The sequences of the ITS2 genes have been deposited in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases. Accession numbers for the species and cultures indicated in Table 2 are as follows: *T. deion*—U76223 (DCLO), U76224 (DLC1), U76225 (DSVP); *T. pretiosum*—U76226 (PRV4), U76227 (PIRV); *T. kaykai*—U76228 (KSH1), U76229 (KLC1); Interior form of *T. platneri*—U76230 (IMSQ).

The size of the *T. kaykai* ITS2 gene is consistently larger than that of either *T. deion* and *T. pretiosum.* The size of the ITS2 gene in order from large to small is: Interior form of *T. platneri* > *T. kaykai* > (*T. deion, T. pretiosum*). The consistent and characteristic differences found in the ITS2 sequences in these four species are reflected in differences in restriction length fragments when the ITS2 gene is restricted with the enzymes MSE1 and ECOR1 as shown in Table 2. The interior form of *T. platneri* differs from the other species in the size of its ITS2 gene (515 bp) and in the presence of the restriction site for ECOR1. *Trichogramma kaykai* differs from both *T. deion* and *T. pretiosum* in the size of its ITS2 gene (470 bp vs 400–410 bp). Also, the ITS2 gene of *T. kaykai* is cut in two large fragments by MSE1 (270 and 200 bp) whereas *T. deion* is cut into three fragments with the largest ca. 300 bp; *T. pretiosum* is not restricted by this enzyme. As with allozymes, the differences between *T. deion* and *T. kaykai* occur in both allopatric and sympatric collections.

Reproductive Data

Reproductive compatibility has frequently been used to support species hypotheses in *Trichogramma* (Pinto and Stouthamer 1994). In this study, four cultures of *T. kaykai* were crossed (at 25°C) with a culture of *T. deion* from Dillon Rd., near Indio, CA (DAW6) and among themselves. The *T. kaykai* cultures used included two from Danby, CA (KDA22, KDA23), and two from Last Chance Cyn., CA (KLC18, KLC21). Crossing procedures employed closely followed those detailed in Pinto et al. (1991). All crosses are based on individual pairings. All combinations of heterogamic crosses were performed concurrently with homogamic controls, resulting in a total of 20 crosses. All crosses included 20 replicates in each direction. For determining relative compatibility between cultures the mean sex ratio (MSR) was calculated as the percentage of female progeny. The relative compatibility of an interculture cross (A × B) is expressed as two percentages: 100% × MSR (A female × B male)/MSR (A female × A male); and the same based on the reciprocal.

Results.—The crosses between cultures of *T. kaykai* and *T. deion* were completely incompatible. Female production is the evidence for reproductive compatibility in arrhenotokous Hymenoptera and not a single female was produced in any of the four heterospecific trials. The mean relative compatibility among the 12 homospecific crosses of *T. kaykai* cultures was 78.0 and ranged from 49.0 to 97.5. The least compatible cross (49.0) was between KDA22 females and KLC21 males. The reciprocal cross was considerably stronger (83.1). There was no evidence that the sympatric cultures (mean compatibility = 74.2, n = 4) were more compatible than the allopatriates (mean compatibility = 79.8, n = 8). In fact, the highest levels (92.6, 97.5) were among allopatric cultures.

Concluding Remarks

Morphologically similar species of *Trichogramma* as exemplified by *T. kaykai* and *T. deion* are apparently quite common (Pinto and Stouthamer 1994). Yet, we feel that in this genus such cryptic species

should be described only after putative morphological differences are shown to be geographically stable and, ideally, found to correlate with other character sources. This requires extensive collecting of all forms involved and an attempt to delineate at least rough geographic distributions. One goal of such work should be the identification of areas of sympatry since it is at such localities that the stability of character differences can be most rigorously tested for. In the case of *T. kaykai*, we have shown that this species has only minor morphological differences from *T. deion* but that these are consistent at several localities including those where the two occur together. Differences in allozymes and the ITS2 sequence, as well as crossing incompatibility, give greater confidence that species recognition is warranted. Thus far we have no evidence of gene flow between these two species. However, a focus of future work should include additional crossing and molecular studies in zones of sympatry. Our efforts in both areas are preliminary.

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